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Impact of spice extracts on the formation of biogenic amines and the physicochemical, microbiological and sensory quality of dry sausage

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ABSTRACT

This study investigated the inhibitory effects of spice (cinnamon, clove, and anise) extracts on the accumulation of biogenic amines (BAs) in Harbin dry sausage. Meanwhile, the physicochemical properties, microorganisms, and sensory quality of dry sausage were evaluated. The results revealed that the accumulation of BAs (cadaverine, putrescine, tyrosamine, 2-phenylethylamine, histamine, and tryptamine) in sausage was significantly inhibited by spice extracts, and cinnamon extract had the best effect. The pH value, water activity, and total volatile basic nitrogen were decreased ($P < 0.05$) and the lipid oxidation was inhibited by spice extracts (especially anise) in dry sausage. The samples with spice extracts had significantly lower total aerobic bacterial counts ($P < 0.05$). Additionally, the spice extracts inhibited the growth of enterobacteriaceae and improved the sensory quality of dry sausage. The results of principal component analysis proved that enterobacteriaceae were responsible for the accumulation of BAs. There was a close positive correlation between the total aerobic bacteria/enterobacteriaceae and BA concentrations. The results revealed that the addition of spice extracts (especially cinnamon) is an advisable method in inhibiting the BA formation, and it can also improve the quality of dry sausage.

1. Introduction

Biogenic amines (BAs) are a type of nitrogenous compound with aromatic, aliphatic, or heterocyclic structures (Wang et al., 2015). BAs are mostly produced through the decarboxylation of amino acids, which are widely present in fermented meat products (Mah & Hwang, 2009; Silla Santos, 1996). When these amines are formed or degraded as part of the normal metabolism *in vivo*, they play significant physiological roles (Morenoarribas & Polo, 2009). BAs may be of endogenous origin at low concentrations in fresh food, such as vegetables and fruit, but high BA concentrations have been found due to uncontrolled microbial enzymatic activity. BAs are by-products of deliberate and accidental bacterial contamination, such as fermented and spoiled food, respectively. An excess of BAs in the food will not only be hazardous to the nervous and cardiovascular systems of human but also lead to changes in food flavour. In addition, BAs are known to be potential precursors of carcinogenic nitrosamines (Lu et al., 2015). For these reasons, the BA content in food is considered an indication of its quality (Li, Bao, Luo, Shen, & Shi, 2012; Shi, Cui, Lu, Shen, & Luo, 2012), as tracked in food processing to monitor microbial contamination levels. BAs, including histamine, putrescine, cadaverine, tyramine, 2-phenylethylamine, and tryptamine, are often detected in fermentation products (Pircher, Bauer, & Paulsen, 2007).

Harbin dry sausage is a traditional fermented meat product in Northeast China that is popular for its unique flavour and texture. However, the traditional processing of dry sausage is still dominated by natural fermentation, which more easily leads to BA formation due to the presence of free amino acids and the growth of microorganisms with decarboxylase activity (Komprda, Sladkova, & Dohnal, 2009). Furthermore, due to their heat stability, once BAs are formed, it is difficult to destroy them with high temperature treatment (Tapingkae, Tanasupawat, Parkin, Benjakul, & Visessanguan, 2010). Microbial contamination in food should be avoided to reduce the production of BAs. Hence, various antimicrobial substances have been used in food to inhibit the accumulation of BAs (Lu et al., 2015; Wang et al., 2015; Özogul, Kacar, & Hamed, 2015).

Spice extracts are natural plant extracts containing aromatic/irritating volatile oils, organic acids, and spicy ingredients. As a substitute for chemical and synthetic preservatives, spice extracts

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are well known for their flavour and antioxidant and antibacterial properties (Srinivasan, 2005), which can be used to inactivate microorganisms such as pathogenic and spoilage bacteria to meet the demands of consumers for food products (Evrendilek, 2015). The antimicrobial properties of spice extracts have been empirically recognized for centuries (Burt, 2004). Spices such as cinnamon, clove, and anise are commonly used in the preservation of meat products for their antimicrobial and antioxidant activities (Krishnan et al., 2014). Evrendilek (2015) found that the essential oils of anise and cinnamon effectively inhibited the growth of 10 bacteria (four Gram-positive bacteria and six Gram-negative bacteria). The research of Passone, Girardi, and Etcheverry (2013) provided further evidence of the anti-fungal and anti-aflatoxigenic properties of cloves. Cinnamon showed high antimicrobial activity in the study by Dussault, Vu, and Lacroix (2014).

Until now, most studies have focused on the antioxidant and antibacterial properties of spice extracts *in vitro* and *in vivo* (Ferrazzano et al., 2011; Nikolova, Petrova, & Zayova, 2013). However, to our knowledge there is little research on the effect of spice extracts on BA in meat products. Therefore, the purpose of this study was to investigate the inhibition of spice (cinnamon, clove, and anise) alcohol extracts on BA accumulation in dry sausage. In addition, the influence of spice extracts on the physicochemical properties, microorganisms, and sensory quality of dry sausage have also been studied.

2. Materials and methods

2.1. Chemicals

Hydrochloric acid, perchloric acid, boric acid, ethanol, methanol, acetonitrile, o-phthaldehyde (OPA), sodium octane sulfonate, Brij-35, glacial acetic acid, anhydrous sodium acetate, boric acid, potassium hydroxide, beta mercaptoethanol, magnesium oxide, methyl red, methylene blue, trichloromethane, trichloroacetic acid, thiobarbituric acid, potassium hydroxide, and sodium hydroxide were purchased from the Solabio Corporation (Beijing, China). The 2-phenylethylamine hydrochloride, putrescine hydrochloride, cadaverine dihydrochloride, histamine, tryptamine, and tyramine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of spice extracts

Spice extracts were prepared as described by Kong, Zhang, and Xiong (2010) with some modifications. Cinnamon, clove, and anise were purchased from a local supermarket (Harbin, China) and dried at 60 °C for 24 h before smashing with an ultrafine pulverizer. Spice powder samples of 50 g were soaked in 95% edible alcohol (400 mL) for 12 h. The solution was suction-filtered, the filter residue was extracted twice, and the combined filtrate was concentrated at 55 °C with a rotary evaporator (Tegent Scientific Ltd., Hong Kong, China) to remove the ethanol. The concentrate was freeze-dried and ground into a powder, which was stored at −20 °C before use.

2.3. Determination of composition of spice extracts

The composition of three spice (cinnamon, clove, and anise) extracts were analyzed using a gas chromatography (7890A-HP5974, Agilent Technologies Co. Ltd., US) coupled to flame ionization detector and mass spectrometry (5975C, Agilent Technologies Co. Ltd., US) using a chromatographic column (HP-5Ms, 0.25 μm, 30.0 m × 0.25 mm) (Diamonsil, Dikma Technologies, Beijing, China) according to the method of Topuz et al. (2016) with some modifications. Samples were extracted with ethanol (2:1, v:v). Determination was carried out at split mode of 10:1. Injection volume and temperature were adjusted to 1 μL and 25 °C, respectively. Helium (99.9%) was the carrier gas with a flow rate of 1.0 mL/min. The oven temperature was programmed as follow: initially temperature was maintained at 60 °C for 5 min, increased at 3 °C/min to 180 °C, and then increased at 10 °C/min to 250 °C. Mass spectra were monitored between 40 and 450 amu and the ionization mode used was electronic impact at 70 eV. Compounds in the extracts were identified by matching their mass spectra and retention times with Wiley, Nist and Flavor libraries. The content of chemical composition of spice extracts was expressed as relative content by area normalization, which is peak area relative to the total peak area %.

2.4. Preparation of dry sausage

Harbin dry sausages were prepared according to a procedure described by Sun, Chen, Li, Zheng, and Kong (2016), with slight modifications. Four groups of dry sausages were prepared. The control group did not contain spice extracts, and the other three groups received alcohol extracts of cinnamon, clove, and anise. Sausages were prepared with lean pork (90%, w/w) and pork back fat (10%, w/w) minced through a 1.5-cm orifice plate with the meat grinder. In addition, sodium chloride (2.5%, w/w), glucose (5%, w/w), sodium nitrite (0.01%, w/w), monosodium glutamate (0.3%, w/w), and wine (1%, w/w) were added.

After thorough mixing, the meat batter was immediately divided into four equal portions (treatments). Three portions of meat batter received an ethanol extract of cinnamon, clove, and anise (0.3 g/kg). The last portion did not receive spice extract (control). Each portion stuffed into a natural porcine casing (3-cm diameter), resulting in sausages with a final weight of approximately 0.15 kg each. Samples were collected during fermentation on days 0, 3, 6, and 9 for analysis. There were 116 sausages in all. For each treatment, there were 29 sausages. For each fermentation time, 6 sausages from each treatment were used to evaluate the pH, moisture content, water activity, thiobarbituric acid reactive substances (TBARS), total volatile basic nitrogen (TVB-N), and bacterial counts. At the end of fermentation, 5 sausages from each treatment were used for a sensory panel evaluation.

2.5. Determination of biogenic amines

Analysis of the BAs by high performance liquid chromatography (HPLC) was performed according to the procedure developed by Latorremoratalla et al. (2009) and Sun et al. (2016). Briefly, 10 g of dry sausage sample was homogenized in 20 mL of 0.4 M perchloric acid. The extract was centrifuged at 4 °C for 10 min at 9000 g. The residue was extracted again under the same conditions. The volume of the combined supernatants was adjusted to 50 mL with 0.4 M perchloric acid. The extract was filtered through a 0.22-μm filter. The derivatization reagent and amine standards were prepared as previously described by Sun et al. (2016) using an HPLC (1100 LC, Agilent Technologies Co. Ltd., US) equipped with a fluorescence detector (Xuzhou HuaiBo instrument and Equipment Co., Ltd., Xuzhou, China) and a C_{18} column (5.0 μm, 250 × 4.6 mm) (Diamonsil, Dikma Technologies, Beijing, China). The test conditions were the same as those described by Sun et al. (2016).

2.6. Determination of pH, moisture content, and water activity

The pH was measured using an electronic pH meter (Mettler Toledo Instruments Co., Ltd., Shanghai, China). The dry sausage (10 g) was homogenized in 90 mL of distilled water and stirred with